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13. ABSTRACT *(Maximum 200*

Intramuscular injection of DNA expression vectors encoding the full length neu cDNA (pNeuN), the neu extracellular domain (pNeuE), or the neu extracellular and transmembrane domain (pNeuTM) can induce protective immunity in FVB/N mice against adoptive transfer of Tg1-1 cells, a *neu* over-expressing tumor cell line generated from a mouse mammary tumor that spontaneously arose in neu transgenic mice. Animal injected with control plasmid DNA could not resist tumor challenge. Anti-neu antibody response of mice immunized with neu expression plasmids were examined by Elisa assay, only some of the animals in pNeuN, pNeuTM, pNeuTM+pIL-2, or pNeuE+pIL-2 treated group were found to develop anti-neu antibodies, the animal in the other group did not develop a detectable anti-neu antibody response 30 days after DNA vaccination.
This year, we examined anti-neu antibody response of DNA vaccine immunized mice again by indirectly flowcytometry assay, we confirm our previous finding. We also examine the cellular immunity response of DNA immunized mice by CTL assay, we find animal in DNA immunized mice did not develop detectable cytotoxic T lymphocyte specific against neu. Additionally, we have cloned and sequenced the mouse neu cDNA sequence from B6 mice.

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Chen ying sep 30 1997

PI - Signature Date

Table of Content

Introduction:

The *neu* oncogene originally was identified by its ability to transform NIH 3T3 cells *in vitro* (1). Mice immunized with *neu*- transformed NIH 3T3 cells developed antibodies reactive with a 185-kDa surface phosphoprotein, that subsequently were found to react with a group I receptor tyrosine kinase encoded by the *neu* oncogene, designated $p185^{neu}$ (2). Subsequently, *neu* was found to be highly homologous to a gene on human chromosome 17 (17q21), designated *erbB-2 (HER-2/neu)* (3).

Over-expression of these proto-oncogenes can lead to neoplastic transformation. *ErbB-2* is over-expressed in 15-40% of all human breast cancers (4, 5, 6, 7). Moreover, over-expression of erbB-2 in breast neoplasms is associated with a poorer survival and a higher risk for recurrent disease after primary therapy (4,8 9, 10, 11,12,13,14). That this association may define a causal relationship is indicated by studies on mice transgenic for the activated or wild-type *neu* proto-oncogene under the control of the mouse mammary tumor virus (MMTV) promoter. Transgenic mice expressing activated-*neu* develop multiple mammary tumors at an early age (15,16). Moreover, transgenic mice with the wild-type *neu* gene under the MMTV promoter also develop focal mammary tumors, albeit with slower kinetics (17). The relative selectivity of erbB-2 overexpression in human adenocarcinomas and the association of erbB-2 and *neu* with a pathogenic mechanism responsible for neoplasia, make the protein product of these genes an attractive target for immunotherapy (18,19).

DNA vaccination is a relatively new technique whereby somatic cells are transfected in vivo with naked plasmid DNA directing synthesis of a target antigen. The expressed protein either can be secreted by the transfected cell or processed inside the cell and presented in the context of MHC antigens. Several studies have shown that such DNA vaccines can induce humoral and/or cellular immune response against the transgene product when injected into skin or muscle(20,21,22,23,24). Moreover, intramuscular injection of DNA encoding human carcinoembryonic antigen (CEA) recently has been found to confer protective immunity against challenge with murine tumor cells transfected to express CEA (24)

We developed *neu* plasmid DNA expression vectors for direct in vivo somatic cell transfection to induce immune responses to the protein product of *neu*. To examine whether DNA expression vectors encoding *neu* could induce protective immunity against tumors cells that over-express this proto-oncogene, we established a tumor cell line, designated Tg1-1, from a *neu*-over-expressing mammary tumor that spontaneously arose in neu transgenic mice. We found that the intramuscular injection of these DNA expression vectors with an expression vector encoding interleukin-2 (pIL-2) can induce protective immunity in FVB/N mice against adoptive transfer of Tg1-1 cells, a neu over-expressing tumor cell line generated from a mouse mammary tumor that spontaneously arose in a FVB/N neu-transgenic mouse. In contrast, animals injected with either control plasmid DNA or pIL-2 alone did not resist tumor challenge. Moreover, co-injection of vectors encoding an immunostimulatory cytokine, IL-2, can augment the efficacy of such DNA vaccines. We examined the anti-neu antibody response of mice immunized with these DNA-expression vector by Elisa assay, we found only some of the animals in pNeuN, pNEUTM, pNeuTM+pIL-2, or pNeuE+pIL-2 treated group develop anti-neu antibodies, the animal in the other group did not develop a detectable anti-neu antibody response 30 days after DNA vaccination.

Recently, I examined anti-neu antibody response of DNA vaccine immunized mice by indirectly flowcytometry assay, I have confirmed my previous finding from Elisa assay.

I also examined the cellular immunity response of DNA immunized mice by CTL assay, I found that mice immunized with neu DNA vaccine did not develop detectable cytotoxic T lymphocyte specific against neu protein .

Other people in our lab recently have compared the efficacy of intradermal versus intramuscular injection of neu expression plasmid in inducing protective immunity against adoptive transfer of a syngenic neu expressing mouse mammary tumor cell line, Tg1-1, in neu transgenic mice. Preliminary data show there is no significantly different in tumor protection between intradermal versus intramuscular injection of neu expression plasmid.

Preliminary data also shown by other people in our lab, that intramuscle co-injection of DNA plasmid which expresses murine CD80 can not enhance the protective immunity induced by neu DNA vaccines agsinst adoptive transfer of Tg1-1 into syngeneic neu-transgenic mice. So I decide not to continue these two part of work.

And we realized that mouse neu cDNA sequence is not available yet, and for further research on gene therapy on breast cancer using mouse or transgenic mouse model, it is necessary to clone and sequence the mouse neu cDNA sequence. For this reason I have cooperated with Dan Hu in our lab to clone and sequence the neu cDNA sequence from B6 mice.

Body:

1. **Evaluate NEU DNA vaccines in FVB/N mice for their ability to induce anti neu antibodies by indirectly flowcytometric analysis.**

In the second annual report , I have use Elisa assay to examined the anti-neu antibody response of mice immunized with neu DNA-expression vector, I found only some of the mice in pNeu N, pNeuTM, pNeuTM+pIL-2, or pNeuE+pIL-2 treated group develop anti neu antibodies, the animal in the other group did not develop a detectable anti-neu antibody response 30 days after DNA vaccination. To confirm this result, recently, I use indirectly flowcytometric analysis to test anti neu antibody.

Method:

1. DNA immunization :

Six to eight week-old FVB/N mice (Jackson, Bar Harbor, ME) were anesthetized with methoxyfluorane. Plasmid DNA (100 mg/injection) suspended in 100 ul of saline was injected into the right quadriceps muscles through a 28-gauge needle at weekly intervals. Mice were bleed via the retro-orbital plexus at weekly intervals to assess for anti-neu antibodies.

2. Production of Adenovirus containing NeuTM and lacZ:

The replication-defective type 5 adenovirus developed by the co-transfection and homologous recombination of the adenoviral transfer vector (ATV) containing NeuTM with the E1 and E3 deleted adenoviral DNA, JM 17. ATV was derived from pE1sp1B introduced with a polyadenylation signal obtained from the restriction enzyme digestion and purification of the XhoI fragment of pRc/CMV. ATV-NeuTM was generated by enzyme digestion and isolation of the NruI-XbaI fragment from pRc/CMV-NeuTM and ligated with the EcoRV-XbaI purified fragment of ATV. The ATV-NeuTM was then co-transfected into 293 cells along with E1 and E3 deleted JM 17 vector. Individual positive recombinant plaques were isolated with a second round of plaque purification performed followed by Cesium Chloride (CsCL) banding. The CsCl was removed from the adenovirus preparation using NAP-20 Sephadex columns. In general, 1X 10^{10} plaque

forming units per milliliter were acquired and a multiplicity of infection value of 10 used for subsequent infection.

3. Indirectly flow cytometry assay for antibodies to neu product:

Indirectly flowcytometric analysis was used to examined for mouse anti-p185 neu antibodies in the sera of control and test animals. For this, We examined whether antisera specifically react with TgRu-1 cells infected with the adeno NeuTM, but not cell infected with adenoLaZ. Both TgRU AdenoNeuTM and TgRuAdenolacZ were removed from the culture flask with 10 mm EDTA in PBS, and washed in FACS buffer (RPMI1640 / 2% fetal calf serum/ 0.1% sodium azide). Approximaly 3X 10^5 cell per analysis were incubated with 1: 20 dilution of antisera or control antiserum at 4 ^oC for 30 minutes. Cell were washed 3 times with FACS buffer then stained 30 minutes at 4 ^oC with the fluorescein isothiocyanate (FITC)-conjugated rat mAB specific for mouse Ig. Cells were washed again and suspended in FACS buffer containing propidiumiodide, and analyzed. For each antisera, we determined the mean fluorescence intensity ratio (MFIR), defined as being the mean fluorescence of TgRU-Adeno NeuTM stained with test antisera versus the mean fluorescence of TgRU adeno NeuTM stained with control antisera. Similarity, We determined the MFIR for each antisera against control of TGRU-Adeno LacZ cells. A positive assay has been define exceeding 2 SD above the mean value of mice which received pCMV control.

Result:

Eight groups of mice were injected with either $pNEU_N$ + pIL-2, $pNEU_{TM}$ + pIL-2, $pNEU_E$ + pIL-2, $pNEU_N$, $pNEU_{TM}$, $pNEU_E$, pIL-2 or pCMV. Each mouse was injected with 100 ug of plasmid DNA at weekly intervals for 4 weeks. Mice were bleed via the retro-orbital plexus at weekly intervals to assess for anti-neu antibodies. Anti-neu antibody response was evaluated by FACS assay in mice immunized with DNA vaccine at 30 days after last DNA immunization . Two mice in pNeuTM treated group developed significant anti-neu antibody titer response 30 days after the final injection of the plasmid DNA , however, the mice in the other groups did not generate a detectable anti-neu antibody response. (Table1.). These result confirm the result from Elisa assay we perform before.

Table 1
Total IgG in mice from experiment 1 and experiment 2 , at 30 days after last DNA immunization.

#	pCMV	pIL-2	$pNEU_N$ + pIL-2	$pNEU_{TM}$ + pIL-2	$pNEU_E$ + pIL-2	$pNEU_N$	$pNEU_{TM}$	$pNEU_E$
1	1.34	1.12	0.89	1.27	0.87	1.46	* 4.87	1.27
2	0.97	1.23	1.53	1.08	1.19	1.05	0.92	1.49
3	1.05	0.81	0.79	1.18	0.90	1.50	1.05	1.02
4	1.39	1.35	0.83	1.64	1.07	0.97	1.26	1.42
5	0.83	0.98	0.87	1.03	0.80	1.16	1.49	0.71
6	1.05	1.36	0.88	0.82	1.14	0.52	* 8.29	1.49
7	1.22	1.08	1.56	1.22	1.23	1.00	3.36	1.51
8	0.91	1.12	0.99	1.60	1.13	0.91	0.98	1.33
Ave	1.09	1.13	1.04	1.23	1.04	1.07	2.77	1.28
S.D.	0.20	0.18	0.31	0.27	0.16	0.31	2.63	0.28

* Anti-neu IgG is higher than mean + 2 x S.D. of 7 mice in pCMV control group.

Similarity , Eight groups of mice were injected with either $pNEU_N$ + pIL-2, $pNEU_{TM}$ + pIL-2, $pNEU_E$ + pIL-2, $pNEU_N$, $pNEU_{TM}$, $pNEU_E$, pIL-2 or pCMV. Each mouse was injected with 100 ug of plasmid DNA at weekly intervals for 4 weeks. Mice were bleed via the retro-orbital plexus at weekly intervals to assess for anti-neu antibodies. Anti-neu antibody response was evaluated by FACS assay in mice immunized with DNA vaccine at 30 days after last DNA immunization . 1 mice in $pNEU_N$ treated group, 4 mice in $pNEU_{TM}$ treated group, 0 mice in $pNEU_E$ treated group, 0 mice in $pNEU_N$ + pIL-2 treated group, 4 mice in $pNEU_{TM}$ + pIL-2 group, and 2 mice in $pNEU_E$ + pIL-2 group demonstrate anti-neu antibody response (Table 2). The different of the mean value MFIR reading between each vaccination and control pCMV and pIL-2 do not reach significant. This result except two sample also confirm the result tested by Elisa assay we perform before.

Table 2
Total IgG in mice from experiment 2, at 30 days after last DNA immunization

#	pCMV	pIL-2	$pNEU_N$ + pIL-2	$pNEU_{TM}$ + pIL-2	$pNEU_E$ + pIL-2	$pNEU_N$	$pNEU_{TM}$	$pNEU_E$
1	1.05	0.99	1.09	1.09	1.05	1.06	*6.68	1.01
2	0.95	1.17	0.92	*3.38	0.97	*2.02	*11.89	0.95
3	0.88	0.85	0.95	0.99	1.12	0.95	1.12	0.94
4	1.01	1.00	0.95	1.07	1.02	1.26	*3.70	1.08
5	1.04	1.05	0.95	*2.43	1.05	1.05	0.97	1.28
6	1.54	0.99	1.19	*3.01	*3.40	1.29	*5.39	0.98
7	1.00	0.92	0.80	*4.51	0.91	1.46		0.71
8	0.98	1.01	1.19	0.97	*1.47	1.23		0.88
Ave	1.05	1.00	1.19	2.18	1.37	1.29	4.95	0.98
S.D.	0.20	0.09	1.00	1.35	0.83	0.33	4.085	0.16
positive	0/8	0/8	0/8	4/8	2/8	1/8	4/6	0/8

* Anti-neu IgG is higher than mean + 2 x S.D. of 6 mice in pCMV control group. (Serum dilution : 1:20)

Taken together of experiment 1 and 2, We found that non of animals in either the pCMV control DNA treated(n=16) or the pIL-2 treated (n=16) developed detectable antibodies against neu 4 weeks after the last DNA immunization. whereas, anti-Neu antibodies were detected in 1 of 16 mice receiving pNeuN, 6/14 mice receiving receiving pNeuTM, 3/16mice receiving pNeuE. surprisingly, these number decreased with the combinatorial vaccination of pNeu cDNA constructs with IL-2: 0/16 mice receiving pNeuN+pIL-2, 4/16 mice receiving pNeuTM+pIl-2, 2/16 mice receiving pNeuE +pIL-2. The number of mice that show positive anti-Neu antibody production was significantly higher in pNeuTM treated group than in pCMV control group ($p< 0.005$, Bonferroni t test). In addition, all mice that develop anti-Neu antibodies in pNeuTM and NeuTM+pIL-2 are tumor free.

II Evaluate neu DNA vaccines in FVB/N mice for their ability to induce cellular immunity against mammary tumor deride from syngenic neu transgenic animals by CTL and Cell Proliferation assay:

Experimental method:

1. Cell lines

To generate mammary tumor cell lines, mammary tumors that spontaneously developed in FVB/N neu transgenic mice were excised, minced into single cell suspensions, and plated in DMEM (Biowhittaker, Walkersville, Maryland), containing 20% fetal bovine serum (FBS), L-glutamine (200 mM, core cell culture facility, UCSD CA), Non Essential Amino Acid (Irvine Scientific, Santa Ana, CA), sodium pyruvate (Irvine Scientific, Santa Ana), and Fungi-bact™ (Irvine Scientific, Santa Ana).

2.Stable transfection of plasmid to Cell line TgRU-1:

The FVB/N syngeneic cell line TgRU-1, from the neu transgenic mice was transfected by $pNEU_N$ and pRcCMV by calcium phosphate assay, as described (25), transfectants were selected in medium containing 0.8 mg/ml of G418 (Gibco, BRL, Grand

Island, NY). Individual G418-resistant colonies were isolated and tested for expression of neu via immunoblotting and flow cytometry .

3.Immunization:

Groups of eight week-old of FVB/N mice receive pNeuTM, pNeuE+pIL-2, pCMV+pIL-2 or pCMV at 100ug/plasmid in 100 ul saline intramuscular injection weekly for 4 weeks.

4. CTL Assay

9-14 days after final injection of plasmid vectors, mice were killed by cervical dislocation, their spleens were removed from mice. In 24 well plate (Costa, Cambridge, MA) 7X10 6 responder splenocytes were incubated with 6 X 10 6 stimulator cell TgRu-NeuN (in the presence of 50 IU /ml recombinant IL-2. The culture media was RPMI1640 supplemented with 10% heated inactivated FBS, 2 mM glutamine, 50 mM 2-mercaptoethanol, and 1% penicillin and streptomycin. The Stimulator cells were irradiated (2000rads). After 5 days, the re stimulated cell were harvested and separated from dead cells on a Lympholyte M (Accurate Chemicals, Westbury, NY) gradient. In 96 well round-bottom plates, target cell were incubated in 200 ul volumes with re stimulated T cell at graded effector to target ratios for 4 h. Assay medium used was phenol red-free RPMI-1640 supplemented with 2% BSA, 2 mm glutamine and 1% penicillin, and streptomycin. The target cell used is TgRU NeuN (describe above), control target is TgRU-pCMV, TgRU-1 (syngenic FVB N mice without expression Neu,) 50 ul of supernatant was then transferred to a 96 well plates and lysis was assessed by measuring lactate dehydrogenase release using the cytotox 96 assay kit (Promega Corp., Madison. WI). Controls were included on each plate for spontaneous LDH release from target and effector cells. Percent lysis was calculated according to the manufacturer's instruction by formula that approximates to:

(test release-spontaneous release)
---------- X100
(maxim release-spontaneous release)

Result:

1.Development of TgRU-1 neu-expressing cell line:

To generate a target cell, we developed a tumor cell line from a mammary tumor that spontaneously developed in a FVB/N neu transgenic mice, designated as TgRU-1. Although derived from neu-transgenic mice, this cell line does not expresses neu protein as assessed by flow cytometry and RT-PCR (data not shown). Preliminary data indicate that this cell line may serve as a sensitive target cell for allogeneic CTL.

We transfected $pNEU_N$ into TgRU-1 to generate a tumor cell line that expresses the neu protein. This cell line, designated TgRU-NEU_N, expresses neu as assessed by flow cytometry (data not shown). We also stable transfected pCMV into TGRU-1 to generate a cell line designatedas TGRU-pCMV. We use TgRU-NEU_N as the positive cell line, and TGRU-pCMV and TgRU-1 as the negative-control target cell, in our CTL assay.

2.CTL

Intramuscular injection of plasmid DNA encoding NEU N, NeuTM or NEU E+IL-2 can not induce detectable neu protein specific CTL in my several experiment. There is no significantly different in % specific lysis to taget cell TgRu-NeuN between splenocyte from

mice immunized with pNeuN, pNeuTM, pNeuE+pIl-2 and control pCMV or pCMV+pIl-2. (See table 3,4,5, 6, 7,8,)

Table 3 **% Specific Lysis of the TgRU-Neu target cell by splenocytes from mice immunized with pNeuN**

		% specific lysis		
	Target	TgRUNeuN	TgRUpCMV	TgRu-1
E:T Ratio	20	15%	13.3%	10.6%
	6	8.3%	7.7%	7.0%
	2	5.4%	4.4%	4.0%

E: effector cell : splenocyte from mice immunized with pNeuN.
T: Target cell: FVB/N syngenic mammary tumor cell line expresses neu protein after stable transfected with pNeuN.

Table 4 **% Specific Lysis of the TgRU-Neu target cell by splenocytes from mice immunized with pNeuTM**

		% specific lysis		
	Target	TgRUNeuN	TgRUpCMV	TgRu-1
E:T Ratio	20	13.10%	10.70%	6.7%
	6	9.2%	7.9%	5.3%
	2	1.2%	1.2%	0.8%

E: effector cell : splenocyte from mice immunized with pNeuTM.
T: Target cell: FVB/N syngenic mammary tumor cell line expresses neu protein after stable transfected with pNeuN.

Table 5 **% Specific Lysis of the TgRU-Neu target cell by splenocytes from mice immunized with pCMV**

		% specific lysis		
	Target	TgRUNeuN	TgRUpCMV	TgRu-1
E:T Ratio	20	9.1%	6.9%	5.0%
	6	8.7%	5.7%	4.4%
	2	5.4%	4.4%	3.2%

E: effector cell : splenocyte from mice immunized with pCMV
T: Target cell: FVB/N syngenic mammary tumor cell line expresses neu protein after stable transfected with pNeuN.

Table 6 **% Specific Lysis of the TgRU-Neu target cell by splenocytes from mice immunized with pNeuE+pIL-2**

		% specific lysis		
	Target	TgRUNeuN	TgRUpCMV	TgRu-1
E:T Ratio	20	12.9%	7.9%	4.4%
	6	8.9%	6.5%	3.7%
	2	3.2%	2.2%	2.35

E: effector cell : splenocyte from mice immunized with pNeuE+pIL-2
T: Target cell: FVB/N syngenic mammary tumor cell line expresses neu protein after stable transfected with pNeuN.

Table 7 % Specific Lysis of the TgRU-Neu target cell by splenocytes from mice immunized with pCMV+pIL-2

		% specific lysis		
	Target	TgRUNeuN	TgRUpCMV	TgRu-1
E:T Ratio	20	9.9%	7.9%	4.4%
	6	6.9%	5.5%	3.7%
	2	2.2%	1.2%	1.35

E: effector cell : splenocyte from mice immunized with pCMV+pIl-2
T: Target cell: FVB/N syngenic mammary tumor cell line expresses neu protein after stable transfected with pNeuN.

Table 8 % Specific Lysis of the TgRU-Neu target cell by splenocytes from mice without immunization

		% specific lysis		
	Target	TgRUNeuN	TgRUpCMV	TgRu-1
E:T Ratio	20	2.0%	2.9%	3.0%
	6	1.4%	3.5%	2.5%
	2	0.2%	0.24%	2.0%

E: effector cell : splenocyte from mice without immunization
T: Target cell: FVB/N syngenic mammary tumor cell line expresses neu protein after stable transfected with pNeuN.

III Another postdoctor in our lab recently has compared the effective of intradermal versus intramuscular injection of neu expression plasmid in inducing protective immunity against adoptive transfer of a syngenic neu expressing mouse mammary tumor cell line, Tg1-1, in neu transgenic mice, preliminary data show there is no significantly different in tumor protection between intradermal versus intramuscular injection of neu expression plasmid.

Preliminary data also shown by other people in our lab, that in vitro somatic cell co-transfection of nake DNA plasmid vectors that direct synthesis of murine CD80 can not enhance the protective immunity induced by neu DNA vaccines agsinst adoptive transfer of Tg1-1 into syngeneic neu-transgenic mice. So I decide not to continue these two part of work.

IV . Cloning and Sequencing of mouse neu cDNA from B6 neanatal mice

We realized that mouse neu cDNA is not available, and for further research on gene therapy on breast cancer using mouse or transgenic mouse model, it is necessary to clone and sequence the mouse neu cDNA sequence. For this reason, by cooperate with Dan Hu in the same lab. I have cloned and sequenced the mouse neu cDNA.

Method and Result:

Neonatal B6 mice was killed, intestine was taken and tissue was homogenized into cell suspension. Total RNA was isolated from cells using 0.5 ml RNAZOL (Tel-Test Inc., Friendswood , TX) per sample, with RNA extracted in chloroform and then precipitated in isopropanal, 1 ug of each cellular total RNA sample was reverse transcribed using superscript TM Reverse Transcriptase and randam primer in Gibco BRL cDNA synthesis kit. We Design 4 pair of neu primer according to the human and rat neu conserved sequence, by doing PCR, We got the 4 cDNA fragment, these four fragment were cloned into the pbluescripts at Hind III site, by sequencing, we got the full length mouse neu cDNA sequence. Finally, we have cloned the full length of mouse neu cDNA fragment into pBluscript by RT-PCR using the primer designed according to the Mouse neu 5' and 3' end sequence which we got from first cloning and sequencing. Below are Mouse neu cDNA sequence. 825-1199 sequence is not available at this time.

1 GGGCCGGAGCCGCAATGATCATCATGGAGCTGGCGGCCTG
41 GTGCCGTTGGGGGTTCCTCC T CGCCCTCCT GTCCCCCGGA
81 GCCGCGGGTA CCCAAGTGTGTACCGGTACC GACATGAAGT
121 TGCGACTCCC TGCCAGTCCT GAGACCCACCTGGACATGCT
161 TCGCCACCTC TACCAGGGCT GTCAGGTGGT GCAGGGCAAT
201 TTGGAGCTTA CCTACCTGCC CGCCAATGCC AGCCTCTCAT
241 TCCTGCAGGA CATCCAGGAA GTCCAGGGAT ACATGCTCAT
281 cGCTCACAAC CGAGTGAAAC ACGTCCCACT GCAGAGGTTG
321CGCATCGTGA GAGGGACTCA GCTCTTTGAG GACAAGTATG
361CCCTGGCTGt GCTAGACAAC CGAGANCCTT TGGAcAACGT
401 CACCGCCGGC CCCCCAGGCA GAACCCCAGA GGGGCTGCGG
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601 GTCCACCTTG TGCCCCAACC TGCAAAGACA ATCACTGTTG
641 GGGTGAGAGT CCTGAAGACT GTCAGATCTT GACTGGCACC
681 ATctGTACTA GTGGCTGTGC CCGGTGCAAG GGCCGGCTGC
721 CCACTGACTG TTGCCATGAG CAGTGTGCTG CAGGCTGCAC
761 GGGTCCCAAG CATTCTGACT GCATGGCCTG CCTCCACTTC
801 AATCATAGTG GTATCTGTGA GCTG..... .....
841 ....
881 ....
921 ....
961 ....
1001 ....
1041
1081 ....
1121 ....
1161..... ....
1201 GAAGCCAGA GCATCTCCAA GTGTTCGAAA CCCTGGAGGA
1241 GATCACAGGT TACCTATACA TCTCAGCATG GCCAGAGAGT
1281 NTCCAAGACC TCAGTGTCTT CCAGAACCTT CGGGTCATTC

1321 GGGGACGGAT TCTCCATGAT GGNGGCTAAT CATTGACGTT
1361 GCAAGGCCTG GGGATCAATT CACTGGGGTA ACGCTCACTG
1401 CGGGAGCTGG GCAGTGGATT GGCTCTCATT CACCGCAACA
1441 CCCATCTTTG CTTTGTAAAC ACTGTACCTT GGGACCAGCT
1481 CTTCCGGAAC CCGCACCAGG CCCTACTCCA CAGTGGGAAC
1521 CGGCCAGAAG AGGNCATGTG NGTCTTGAGN GGCTTGGTCT
1561GTAACTCACT GTGTGCCCGT GGGCACTGCT GGGGGCCAGG
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1641CAGGAGTGTG TGGAGGAGTG CCGAGTATGG AAGGGGCTCC
1681CCAGGGAGTA TGTGAGGGGC AAGCACTGTC TGCCATGCCA
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1801 AGGACTCATC TTCCTGTGTG GCTCGCTGCC CCAGTGGTGT
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1921CCCACTCATG TGTGGACCTG GACGAACGAG GCTGCCCACC
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2401 CATCCACAGT GCAGCTGGTG ACACAGCATA TGCCCTATGG
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2801 TGGGATCCCA GCTCGGGAGA TCCCTGATTT GCTGGAGAAG
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3441 GAGTATGTGA ACCAGCCAGA GGTTCGGCCT CAGTCTCCCT
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3561 AATGGGGTTG TCAAAGACGT TTTTGCCTTT GGGGGTGCTG
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3681 GACAACCTCT ATTACTGGGA CCAGAACTCA TCGGAGCAGG
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4001 AACAGCACAC TGTTCAGCCC CAGAGGATTA CAGACCCTGA
4041CTGCCCTGAC AGACTGTAGG GTCCAGTGGG TATTCCTTAC
4081CTGG

Conclusion:

Intramuscular injection of DNA-expression vectors encoding the full length neu cDNA ($pNEU_N$), the neu extracellular domain ($pNEU_E$), or the neu extracellular and transmembrane domains ($pNEU_{TM}$) can induce protective immunity in FVB/N mice against adoptive transfer of Tg1-1, a syngeneic neu-expressing mammary tumor that spontaneously arose in neu-transgenic mice. In contrast, animals injected with either control plasmid DNA or a DNA-expression vector encoding interleukin-2 (pIL-2) could not resist tumor challenge. I examined the anti-neu antibody response of mice immunized with these DNA-expression vectors. Although some of the animals in the $pNEU_{TM}$, $pNEU_{TM}$ + pIL-2, or $pNEU_E$ + pIL-2 treated groups did develop anti-neu antibodies, the animals in the other groups did not develop a detectable anti-neu antibody response, even at 30 days after DNA vaccination. We confirm this finding by examining anti-neu antibody response of DNA vaccine immunized mice by indirectly flowcytometry assay. I also find mice immunized with neu DNA vaccine did not develop detectable cytotoxic T lymphocyte specific against neu protein. In addition, I have cloned and sequence the mouse neu cDNA from B6 mouse.

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2.**Ying Chen,** Dan Hu, David Eling, Joan Robbins, Thomas J. Kipps. DNA Vaccines Encoding Full-length or Truncated Neu Induce Protective Immunity Against Neu-Expressing Mammary Tumor (Submitted to Cancer Research)

Abstract:

1.Saraswati Sukumar. Katherine E. Mckenzie, **Ying Chen,** Prem.M Sharma, and Marrianne Bowman, Chemically-induced tumor in rodens as model systems for genetic analysis of human cancer . International workshop on receptor mediated mechanism in chemical carcinogenesis Lyon France 14-16 Nov 1994

2.Dan Hu, **Ying Chen,** Joan Robbins, Thomas J. Kipps. Erb-2/neu DNA Vaccines Based Immunotherapy. The Nineth International Congress of Immunology July 23-29 1995 San Francisco

3.**Ying Chen,** Dan Hu, Joan Robbins, Thomas J. Kipps. Antitumor immunity elicited by polynucleotide vaccines encoding full-length or truncated membrane bound or secreted forms of erbB-2/neu.

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